

CLAIMS

sub C17 1. A fluorescent protein modified such that said modified fluorescent protein incorporates a cleavage site for a protease, cleavage of said modified fluorescent protein at said cleavage site by said protease causing the alteration of at least one of the emission and excitation spectra of said modified fluorescent protein.

2. A fluorescent protein according to claim 1, being a green fluorescent protein.

sub p27 3. A fluorescent protein according to claim 2, said modified fluorescent protein having said cleavage site incorporated in the loop structure joining any pair of adjacent  $\beta$ -sheets.

sub C2 4. A fluorescent protein according to claim 3, said pair of adjacent  $\beta$ -sheets being selected from the group consisting  $\beta$ -sheets numbers 9 and 10, 5 and 6, and 8 and 9.

5. A fluorescent protein according to claim 3, said modified fluorescent protein being selected from any one of the group consisting D9, MD9, D4, D7, D8, E2, E3-1, E3-5, E3-9, E3-12, E4-a, E4-g, E4-j, E4-o and E4-p.

6. A fluorescent protein according to claim 1, being selected from any one of the group consisting BFP, CFP, YFP and DsRed.

7. A fluorescent protein according to claim 1, said cleavage site having the sequence of any one of the group consisting SEQ ID NOs: 4 and 7-13.

8. A nucleic acid sequence encoding a fluorescent protein according to claim 1.
9. A recombinant DNA construct comprising a regulatory element operatively linked to a nucleic acid sequence according to claim 8.
10. A cell transformed or transfected with a recombinant DNA construct according to claim 9.
11. A method of determining protease activity in a sample cell transformed or transfected according to claim 10, comprising the steps of:
- i) determining at least one of the emission and excitation spectra of said sample cell;
  - ii) determining for a cell transformed or transfected according to claim 9 and having a known protease activity the emission and/or excitation spectra determined in step (i);
  - iii) comparing the result of detection steps (i) and (ii); and
  - iv) correlating the results of comparison step (iii) to determine the level of said protease activity in said sample cell.
12. A method of detecting protease activity in a sample cell according to claim 11, the cell having a known protease activity having no protease activity.
13. A method of detecting a change in protease activity in a sample cell transformed or transfected according to claim 10, comprising the steps of:
- i) at a first timepoint, determining at least one of the emission and excitation spectra of said sample cell;

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- ii) at a second timepoint, re-determining the emission and/or excitation spectra of said sample cell determined in step (i);
- iii) comparing the results of steps (i) and (ii); and
- iv) correlating the results of comparison step (iii) to determine any change in said protease activity in said sample cell.

14. A method of determining the effect of a compound on the activity of a protease in a cell transformed or transfected according to claim 10, comprising the steps of:

- i) determining at least one of the emission and excitation spectra of said cell;
- ii) treating said cell with said compound and re-determining the emission and/or excitation spectra of said cell determined in step (i);
- iii) comparing the results of steps (i) and (ii); and
- iv) correlating the results of comparison step (iii) to determine the effect of said test agent upon said activity of said protease.

15. A method of determining the effect of a compound on the activity of a protease in first and second cells transformed or transfected according to claim 10, comprising the steps of:

- i) determining at least one of the emission and excitation spectra of said first cell;
- ii) treating said second cell with said compound and determining the emission and/or excitation spectra of said second cell determined in step (i) for said first cell;
- iii) comparing the results of steps (i) and (ii); and
- iv) correlating the results of comparison step (ii) to determine the effect of said compound upon said activity of said protease.

16. A method of determining protease activity in a sample, comprising the steps of:

- i) adding to said sample a fluorescent protein according to claim 1 and the conditions necessary to allow fluorescence, and determining at least one of the emission and excitation spectra of said sample;
- ii) adding to a control sample a fluorescent protein according to claim 1 and the conditions necessary to allow fluorescence, and determining the emission and/or excitation spectra determined in step (i), the control sample having a known protease activity;
- iii) comparing the result of detection steps (i) and (ii); and
- iv) correlating the results of comparison step (iii) to determine the level of said protease activity in said sample.

17. A method of detecting protease activity in a sample according to claim 16, the control sample not having any protease activity.

18. A method of detecting a change in protease activity in a sample, comprising the steps of:

- i) adding to said sample a fluorescent protein according to claim 1 and the conditions necessary to allow fluorescence;
- ii) at a first timepoint, determining at least one of the emission and excitation spectra of said sample;
- iii) at a second timepoint, re-determining the emission and/or excitation spectra of said sample determined in step (i);
- iv) comparing the results of steps (ii) and (iii); and
- v) correlating the results of comparison step (iv) to determine any change in the level of said protease activity in said sample.

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19. A method of determining the effect of a compound on the activity of a protease in a sample, comprising the steps of:

- i) adding to said sample a fluorescent protein according to claim 1 and the conditions necessary to allow fluorescence;
- ii) determining at least one of the emission and excitation spectra of said sample;
- iii) treating said sample with said compound and re-determining the emission and/or excitation spectra of said sample determined in step (ii);
- iv) comparing the results of steps (ii) and (iii); and
- v) correlating the results of comparison step (iv) to determine the effect of said test agent upon said activity of said protease.

20. A method of determining the effect of a compound on the activity of a protease in first and second samples, comprising the steps of:

- i) adding to said samples a fluorescent protein according to claim 1 and the conditions necessary to allow fluorescence;
- ii) determining at least one of the emission and excitation spectra of said first sample;
- iii) treating said second sample with said compound and determining the emission and/or excitation spectra of said second sample determined in step (ii) for said first sample;
- iv) comparing the results of steps (ii) and (iii); and
- v) correlating the results of comparison step (iii) to determine the effect of said compound upon said activity of said protease.

21. The use of a cell according to claim 10 in a method of determining the activity of said protease.

22. The use of a protein according to claim 1 in a method of determining the activity of said protease.

23. The use of a protein according to claim 1 in a method of determining the effect of a compound on the activity of said protease.

24. The use of a protein according to claim 1 in a method of determining the effect of first and second compounds on the activity of said protease.

25. A fluorescent protein according to claim 1, said protease being a caspase.

26. A fluorescent protein according to claim 25, said caspase being selected from any one of the group of caspase-3, caspase-8 and caspase-9.

27. A method according to any one of claims 11-15, said protease comprising a caspase and said method comprising a method of detecting apoptosis of said sample cell.

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